

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:)	Group Art Unit 1809
)	
Hogan et al.)	Examiner MARSCHEL, A
)	
Serial No. 08/454,529)	
)	
Filed: May 30, 1995)	
)	
For: NUCLEIC ACID PROBES FOR)	
DETECTION AND/OR QUANTITATION)	
OF NON-VIRAL ORGANISMS)	
)	

Proposed Claims

A. (Proposed) A method of detecting whether one or more target species may be present in a sample comprising the steps of:

(a) contacting said sample with means for detecting if a nucleic acid variable region characteristic of said one or more target species may be present in said sample, wherein said means distinguishes a nucleic acid variable region characteristic of said one or more target species from the nucleic acid of least one nontarget species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;
bases 125-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-635 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;

bases 980-1050 of *E. coli* 16S rRNA or the encoding DNA;
bases 1255-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 540-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2195-2235 of *E. coli* 23S rRNA or the encoding DNA; and

b) determining if said means detects the presence of said nucleic acid variable region characteristic of said one or more target species as an indication that said one or more target species may be present in said sample.

B. (Proposed) A method of detecting whether one or more target species may be present in a sample comprising the steps of

a) contacting said sample with an oligonucleotide probe which distinguishes between nucleic acid of said one or more target species from nucleic acid of at least one nontarget species, said oligonucleotide probe made by a process comprising the following steps:

i) identifying a variable region present in said nucleic acid of one or more target species and present in said nucleic acid of at least one non-target species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a target region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;
bases 125-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-635 of *E. coli* 16S rRNA or the encoding DNA;

bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1050 of *E. coli* 16S rRNA or the encoding DNA;
bases 1255-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 540-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2195-2235 of *E. coli* 23S rRNA or the encoding DNA; and

ii) producing said oligonucleotide probe to comprise a target-complementary sequence, wherein said target-complementary sequence is obtained by substantially maximizing complementarity to said variable region present in said one or more target species, while substantially minimizing complementarity to said variable region present in said at least one non-target species, such that a duplex formed between said oligonucleotide probe and said nucleic acid of one or more target species has a higher T_m than a duplex formed between said oligonucleotide probe and said nucleic acid of at least one non-target species; and

b) detecting the presence a nucleic acid complex comprising said oligonucleotide probe formed under conditions of high stringency as an indication that said one or more target species may be present in said sample, wherein under said conditions said oligonucleotide probe does not form a detectable duplex with said nucleic acid of at least one non-target species.

C. (Proposed) A method of detecting whether one or more target species may be present in a sample comprising the steps of:

(a) contacting said sample with means for detecting if a nucleic acid variable region characteristic of said one or more target species may be present in said sample, wherein said means distinguishes a nucleic acid variable region characteristic of said one or more target

species from the nucleic acid of least one nontarget species belonging to the same genus as said one or more target species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;
bases 125-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-635 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1050 of *E. coli* 16S rRNA or the encoding DNA;
bases 1255-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 540-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2195-2235 of *E. coli* 23S rRNA or the encoding DNA; and

b) determining if said means detects the presence of said nucleic acid variable region characteristic of said one or more target species as an indication that said one or more target species may be present in said sample.

D. (Proposed) A method of detecting whether one or more target species may be present in a sample comprising the steps of

a) contacting said sample with an oligonucleotide probe which distinguishes between nucleic acid of said one or more target species from nucleic acid of at least one nontarget species belonging to the same genus as said one or more target species, said oligonucleotide probe made by a process comprising the following steps:

i) identifying a variable region present in said nucleic acid of one or more target species and present in said nucleic acid of at least one non-target species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;
bases 125-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-635 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1050 of *E. coli* 16S rRNA or the encoding DNA;
bases 1255-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 540-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and
bases 2195-2235 of *E. coli* 23S rRNA or the encoding DNA; and

ii) producing said oligonucleotide probe to comprise a target-complementary sequence, wherein said target-complementary sequence is obtained by substantially maximizing complementarity to said variable region present in said one or more target species, while substantially minimizing complementarity to said variable region present in said at least one non-target species, such that a duplex formed between said oligonucleotide probe and said nucleic acid of one or more target species has a higher T_m than a duplex formed between said oligonucleotide probe and said nucleic acid of at least one non-target species; and

b) detecting the presence a nucleic acid complex comprising said

oligonucleotide probe formed under conditions of high stringency as an indication that said one or more target species may be present in said sample, wherein under said conditions said oligonucleotide probe does not form a detectable duplex with said nucleic acid of at least one non-target species.

E. (Proposed) A method of detecting whether one or more target species may be present in a sample comprising the steps of:

(a) contacting said sample with means for detecting if a nucleic acid variable region characteristic of two or more non-viral target species belonging to a first genus, one of which is said one or more target species, from at least one non-viral nontarget species belonging to a second genus, wherein said means distinguishes said nucleic acid variable region characteristic of said two or more non-viral target species from the nucleic acid of said least one nontarget species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a region selected from the group consisting of:

bases 65-108 of *E. coli* 5S rRNA or the encoding DNA;
bases 60-105 of *E. coli* 16S rRNA or the encoding DNA;
bases 125-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-490 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-635 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-870 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1050 of *E. coli* 16S rRNA or the encoding DNA;
bases 1255-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-405 of *E. coli* 23S rRNA or the encoding DNA;
bases 540-575 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and

bases 2195-2235 of *E. coli* 23S rRNA or the encoding DNA; and

b) determining if said means detects the presence of said nucleic acid variable region characteristic of two or more non-viral target species belonging to a first genus as an indication that said one or more target species may be present in said sample.

F. (Proposed) A method for detecting whether a target species may be present in a sample comprising the steps of:

a) contacting said sample with an oligonucleotide probe able to distinguish two or more non-viral target species belonging to a first genus, one of which is said target species, from at least one non-viral nontarget species belonging to a second genus, said oligonucleotide probe made by a process comprising the following steps:

i) aligning a variable region present in each of said two or more target species and in said one or more nontarget species, wherein said variable region is present in an rRNA sequence, or a DNA sequence encoding for said rRNA sequence, in a location corresponding to a region selected from the group consisting of:

bases 60-100 of *E. coli* 16S rRNA or the encoding DNA;
bases 120-150 of *E. coli* 16S rRNA or the encoding DNA;
bases 170-230 of *E. coli* 16S rRNA or the encoding DNA;
bases 405-480 of *E. coli* 16S rRNA or the encoding DNA;
bases 600-670 of *E. coli* 16S rRNA or the encoding DNA;
bases 820-860 of *E. coli* 16S rRNA or the encoding DNA;
bases 980-1050 of *E. coli* 16S rRNA or the encoding DNA;
bases 1250-1290 of *E. coli* 16S rRNA or the encoding DNA;
bases 270-390 of *E. coli* 23S rRNA or the encoding DNA;
bases 535-560 of *E. coli* 23S rRNA or the encoding DNA;
bases 1150-1200 of *E. coli* 23S rRNA or the encoding DNA;
bases 1440-1600 of *E. coli* 23S rRNA or the encoding DNA;
bases 1710-1750 of *E. coli* 23S rRNA or the encoding DNA; and

bases 2190-2330 of *E. coli* 23S rRNA or the encoding DNA; and

ii) substantially maximizing complementarity of said nucleotide sequence to said variable region present in each of said two or more target species while substantially minimizing the complementarity of said nucleotide sequence to said variable region present in said at least one non-viral nontarget species, such that a probe:target duplex formed between said oligonucleotide probe and nucleic acid of each of said two or more target species has a higher T_m than a duplex formed between said oligonucleotide probe and said nucleic acid of at least one nontarget species; and

b) detecting the presence a nucleic acid complex comprising said oligonucleotide probe formed under conditions of high stringency as an indication that said one or more target species may be present in said sample, wherein under said conditions said oligonucleotide probe does not form a detectable duplex with said nucleic acid of at least one non-target species.